



(19) Europäisches Patentamt
European Patent Office
Office européen des brevets



(11) EP 0 441 763 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
21.05.1997 Bulletin 1997/21

(51) Int Cl. 6: C12N 15/16, C12P 21/02,
C07K 14/475

(21) Application number: 91870003.0

(22) Date of filing: 07.01.1991

(54) Heparin-binding growth factor

Heparin bindender Wachstumsfaktor

Facteur de croissance liant l'héparine

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(30) Priority: 08.01.1990 US 462156
21.06.1990 US 542232

(43) Date of publication of application:
14.08.1991 Bulletin 1991/33

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Description

This invention relates to a novel peptide growth factor and, more particularly, to a heparin-binding growth factor derived from human placenta.

5 In recent years a considerable number of growth factors derived from various animal cells have been isolated and characterized. Illustrative of these growth factors are nerve growth factor (NGF) which has been purified from several different cell sources, insulin-like growth factors (IGF-I and IGF-II), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), endothelial cell growth factor (ECGF), somatomedins and transforming growth factors (TGF) derived from various tumors and virally transformed cells. For background information 10 on these growth factors see, for example, the recent brief review articles by Kris et al., Biotechnology 3, 135-140 (1985); Dijk and Iwata, Ibid. 7, 793-798 (1989); and the comprehensive review in Hormonal Proteins and peptides, Ed. by Choh Hao Li, Vol. 12, "Growth Factors," Academic Press, 1984.

15 Although the existence of acidic and basic fibroblast growth factor (aFGF and bFGF) has been known for fifty years [Trowell, et al., J. Exp. Biol. 16, 60-70, (1939); Hoffman, Growth 4, 361-376 (1940)], only recently have they been purified and sequenced [Thomas et al., Proc. Natl. Acad. Sci. USA 81, 357-361 (1984); Gimenez-Gallego et al., Science 230, 1385-1388 (1985); Lemmon and Bradshaw, J. Cell. Biochem. 21, 195-208 (1983); Bohlen et al., Proc. Natl. Acad. Sci. USA 81, 5364-5368 (1984); and Esch et al., Proc. Natl. Acad. Sci. USA 82, 6507-6511 (1985)]. aFGF and bFGF have 55% sequence homology, suggesting that they arose by duplication and divergence from a common ancestral gene. Cells responding to the FGFs have between 10^3 and 10^5 high affinity receptors per cell [Neufeld and Gospodarowicz, J. Biol. Chem. 261, 5631-5637 (1986)]. The bFGF receptor has recently been purified, sequenced, and cloned [Lee et al., Science 245, 57-60 (1989)]. Both bFGF and aFGF compete for the same receptor and displace each other in radio-receptor assay [Neufeld, supra; Olwin and Hauschka, Biochemistry 25, 3487-3492 (1986)]; however, two forms of the receptor appear to have different affinities for aFGF and bFGF. Recently five additional proteins have 20 been reported by cDNA cloning to have homology to the FGFs (hst, int-2, FGF-5, FGF-6, and KGF or FGF-7) [Dickson and Peters, Nature 326, 833 (1987); Yoshida et al., Proc. Natl. Acad. Sci. USA 84, 7305-7309 (1987); Delli Bovi et al., Cell 50, 729-737 (1987); Zhan et al., Mol. Cell. Biol. 8, 3487-3495 (1988); Marcis et al., Oncogene 4, 335-340 (1989); and Finch et al., Science 245, 752-755 (1989)]. All five cDNA sequences encode signal peptides, therefore suggesting 25 that these five proteins are presumably secreted, unlike aFGF or bFGF which lack signal peptides.

30 In accordance with the present invention a novel heparin-binding growth factor has been isolated from human placenta. This novel heparin-binding growth factor, herein also designated as HBGF-8, is a 18.9 kDa polypeptide with a unique 25 N-terminal amino acid sequence as follows:

HBGF-8

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Gly-Lys-Lys-Glu-Lys-Pro-Glu-Lys-Lys-Val-Lys-Lys-Ser-Asp-

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Cys-Gly-Glu-Trp-Gln-Trp-Ser-Val-Cys-Val-Pro.

This novel growth factor binds tightly to cation exchange resins and to Heparin-Sepharose® and is stable to acetone precipitation and labile in acid.

45 HBGF-8 was as active as acidic fibroblast growth factor (aFGF) and slightly less active than bFGF in the mouse NIH 3T3 fibroblast mitogenic assay system with an intrinsic specific activity of 5000 dpm/ng under standard assay conditions.

50 In an illustrative example and based upon total activity in the acetone extracts of bovine uterus stimulating 3 H-thymidine incorporation into DNA of serum-starved NIH 3T3 cells, a 6940 fold purification was achieved with an overall yield of HBFG-8 activity of 0.4%, using extraction of acetone powders and chromatographic separations at neutral pH. Approximately 18 μ g protein was obtained from 1.2 kg wet weight of tissue. HBGF-8 was clearly separated from 17.5 kDa bovine uterus basic fibroblast growth factor (bFGF) by purification and its N-terminal amino acid sequence analysed. The unique sequence as set forth above was found.

55 In accordance with another aspect of the invention, the complete coding sequence of cDNA clones representing the full size human HBGF-8 have been developed.

The human HBGF-8 cDNA sequence was isolated from human placenta cDNA library using bovine HBGF-8 cDNA fragment as a probe. The bovine HBGF-8 cDNA sequence was isolated from bovine uterus cDNA library using the above HBGF-8 N-terminal sequences as a probe. The cDNA sequence contains 995 nucleotides and encodes a 18.9 kDa (M_r = 18,942) protein of 168 amino acids, including a 32 amino acid lead sequence. The cDNA coding sequence

starts at nucleotide position 252 to 755.

Comparison of the bovine and human HBGF-8 showed that HBGF-8 is highly conserved. Of 168 amino acids, 163 were identical. Differences occur at amino acid positions 3, 4 and 7 in the leader sequence and at amino acid positions 130 and 147 in the mature protein of the complete 168 amino acid sequence.

5 The human and bovine cDNA protein sequences and the human and bovine cDNA sequences for HBGF-8 are as follows:

Human HBGF-8 Protein

10	Met Gln Ala Gln Gln Tyr Gln Gln Gln Arg Arg Lys Phe Ala Ala	15
15	Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala	30
20	Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser	45
25	Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly	60
30	Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu	75
35	Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn	90
40	Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala	105
45	Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly	120
50	Ser Leu Lys Arg Ala Leu His Asn Ala Glu Cys Gln Lys Thr Val	135
55	Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Pro Lys Pro Gln	150
60	Ala Glu Ser Lys Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys	165
65	Met Leu Asp	168

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Bovine HBGF-8 Protein

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Met Gln Thr Pro Gln Tyr Leu Gln Gln Arg Arg Lys Phe Ala Ala	15
Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala	30
Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser	45
Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly	60
Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu	75
Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn	90
Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala	105
Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly	120
Ser Leu Lys Arg Ala Leu His Asn Ala Asp Cys Gln Lys Thr Val	135
Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Ser Lys Pro Gln	150
Ala Glu Ser Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys	165
Met Leu Asp	168

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HUMAN HBGF-8 cDNA

5	GTCAAAGGCA GGATCAGGTT CCCCCGCTTC CAGTCCAAAA ATCCCGCCAA	50
	GAGAGCCCCA GAGCAGAGGA AAATCCAAAG TGGAGAGAGG GGAAGAAAGA	100
10	GACCAGTGAG TCATCCGTCC AGAAGGCGGG GAGAGCAGCA GCGGCCAAG	150
	CAGGAGCTGC AGCGAGCCGG GTACCTGGAC TCAGCGGTAG CAACCTCGCC	200
15	CCTTGCAACA AAGGCAGACT GAGCGCCAGA GAGGACGTTT CCAACTCAA	250
	AATGCAGGCT CAACAGTACC AGCAGCAGCG TCGAAAATTT GCAGCTGCCT	300
20	TCTTGGCATT CATTTCATA CTGGCAGCTG TGGATACTGC TGAAGCAGGG	350
	AAGAAAGAGA AACCAAGAAAA AAAAGTGAAG AAGTCTGACT GTGGAGAATG	400
25	GCAGTGGAGT GTGTGTGTGC CCACCACTGG AGACTGTGGG CTGGGCACAC	450
	GGGAGGGCAC TCGGACTGGA GCTGAGTCCA AGCAAACCAT GAAGACCCAG	500
30	AGATGTAAGA TCCCCTGCAA CTGGAAGAAG CAATTTGGCG CGGAGTGCAA	550
	ATACCAGTTC CAGGCCTGGG GAGAATGTGA CCTGAACACA GCCCTGAAGA	600
35	CCAGAACTGG AAGTCTGAAG CGAGCCCTGC ACAATGCCGA ATGCCAGAAG	650
	ACTGTCACCA CTCCCAAGCC CTGTGGAAA CTGACCAAGC CCAAACCTCA	700
	AGCAGAAATCT AAGAAGAAGA AAAAGGAAGG CAAGAAACAG GAGAAGATGC	750
	TGGATTAAAA GATGTCACCT GTGGAACATA AAAAGGACAT CAGCAAACAG	800
40	GATCAGTTAA CTATTGCATT TATATGTACC GTAGGCTTG TATTCAAAAA	850
	TTATCTATAG CTAAGTACAC AATAAGCAAA AACAAAAAGA AAAAAAAA	900
	AAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA 950	
	AAAAAAA A	961

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Bovine HBGF-8 cDNA

5	GAGTGGAGAG	AGTAGAAGAA	AGAGAGCAGG	GAGTCACCGG	GCGTGCGGGG	50
	GCGGAGAGCA	GCGGCCGCCG	CGAGCACCAAG	CGACTTGGGT	ACCTGGACTC	100
10	AGGGCAGAAA	AACCTCTCCC	GGATCAACAA	AGGCAGCCTG	AGCGCGCACC	150
	GCTCTTTGC	GA CT CCAAAA	TGCAGACTCC	ACAGTACCTG	CAGCAACGTC	200
15	GAAAATTG	GC	AGCTGCC	TTT	GGCAGCTGTG	250
	GACACCGCTG	AAGCAGGAAA	GAAAGAGAAA	CCAGAAAAGA	AGGTGAAGAA	300
20	GTCTGACTGT	GGAGAATGGC	AGTGGAGTGT	GTGTGTACCA	ACCAGTGGGG	350
	ACTGTGGGCT	GGGCACCCGC	GAGGGCACCC	GTACCGGAGC	TGAGTGTAAA	400
25	CAAACCATGA	AGACCCAGAG	ATGTAAGATC	CCCTGCAACT	GGAAAAAGCA	450
	ATTTGGAGCG	GAGTGCAAAT	ACCAGTTCCA	GGCCTGGGGA	GAATGTGATC	500
30	TGAACACGGC	TCTGAAGACC	CGAACTGGGA	GCCTGAAGCG	AGCCCTCCAC	550
	AACGCCGACT	GCCAGAAGAC	AGTCACCATC	TCCAAGCCCT	GTGGCAAGCT	600
35	GACCAAGTCC	AAACCTCAAG	CAGAATCTAA	GAAGAAGAAA	AAGGAAGGCA	650
	AGAAACAGGA	GA AG GATGCTG	GA CT AAAAGC	CACCACCTTC	CGTGGACCAT	700
40	GAAAAGGACA	TCAGCAAACA	CGATCAGTTA	ACTATTGCAT	TTATATCTAC	750
	CGTAGGCTTT	TTATTCAAAA	ATTATCTATA	GCTTAAGTAC	ACAATAGGCA	800
45	GAAACAAAAT	GAAAAGAAAA	ATTTTGTAGT	AGCATT	TTAAATGTAT	850
	CAATATACCA	TAGTACCACT	AGGGACTTAT	AATAGAGGAC	TGTAATCCTA	900
50	TTTAGAATGT	TGACTTATAG	TACATGTTAA	GTGATAGAAA	ACTGAGGTAA	950
	GT TTTTTGAA	GT TTATGTGAT	ATTTTACATT	ACATTTTTT	TTACATT	1000
	TTCTTTGGC	AGCAATTAA	ATGTTATGAC	TATGTA AA CT	ACTTCTCTTG	1050
	TTAGGTAATT	TTTTCACCT	AGACTTTATT	TCCCAATTGA	GAAAATATC	1100
	TACTAAACAA	AGCAGCAATA	AAATATGATC	ATCCTATCTG	AGGAAAATAT	1150
	CTCTTTCT	GCCAGTGGAT	TTTAAAAAA	TTGTAGTC	CAAAT	1196

In the bovine HBGF-8 protein sequence, the following amino acid replacements of the human sequence are found:

55	Ala-3-Thr; Gln-4-Pro; Gln-7-Leu; Glu-130-Asp;
	and Pro-147-Ser.

Although the present invention is directed to a heparin-binding growth factor derived from human placenta, it is considered that assistance in understanding the invention will be obtained from the following detailed description of heparin-binding growth factor derived from bovine uterus taken in connection with the accompanying drawings in which briefly:

5 FIG. 1 is a graphical representation which shows Chromatographic Separation of Bovine Uterus Mitogenic Activity. The mitogenic activity that bound to Heparin-Sepharose between 900 mM and 2000 mM NaCl was concentrated from 1160 ml to 12 ml using a MonoS HR 10/10 column, elution of this column using 2000 mM NaCl, and reapplication following dilution to 500 mM NaCl to the MonoS HR 5/5 column shown above. The A_{280} absorption profile elution of protein is shown by the shaded area. The results of the solid phase ELISA to detect bovine bFGF immunoreactivity are shown by the open triangles. In addition, the mitogenic activity in the mouse NIH 3T3 assay system is shown by the open squares. The NaCl gradient that was applied from 500 mM to 2000 mM salt is shown by the dashed line and individual fractions of 1 ml were collected. The bFGF immunoreactivity is expressed as μ g/ml of bFGF. The region of the column shown by the continuous line and labeled region A was pooled, dialyzed, concentrated and applied to the microbore cation exchange column.

10 15 FIG. 2 is a graphical representation which shows cation exchange chromatographic separation of mitogenic proteins from bovine uterus. Region A (Fig. 1) was applied to a 2.1 x 220 nm Aquapore CX-300 cation exchanger and a 0-2 M NaCl gradient applied. The gradient is shown by the dashed line and the elution pattern of the proteins or A_{280} by the continuous line; the histogram shows the mitogenic activity of the fractions in the NIH 3T3 assay system. The HBGF-8 peak of mitogenic activity can be seen to elute at molarity of 1-1.2 M salt.

20 FIG. 3 shows the SDS-PAGE of the pure bovine uterus bFGF and HBGF-8. Lane 1 shows the appearance after SDS-PAGE in 15% gels of the pure 17.5 kD bovine uterus bFGF following reverse phase C4 chromatographic purification. This material was used for N-terminal sequencing. Lane 2 shows the appearance of the 17 kD novel HBGF-8 in the same gel system. This tube was used for determining novel N-terminal amino acid sequence shown above. The molecular weight markers used were BRL low molecular weight range markers.

25 In order to illustrate specific preferred embodiments of the invention in greater detail, the following exemplary laboratory preparative work was carried out. It will be appreciated, however, that the invention is not limited to these specific examples or the details described therein.

EXAMPLE 1

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METHODS

35 Mitogenic Assays. The mitogenic activity of samples was assayed in triplicate by measuring the incorporation of [$\text{methyl-}^3\text{H}$] thymidine into acid-insoluble DNA of confluent, serum-starved NIH 3T3 cells. Unstimulated cells were used as background and values subtracted as background, which averaged 1500 dpm.

40 Immunological Methods. Rabbit anti-bFGF polyclonal antisera were obtained from Dr. Judith Abraham (California Biotechnology, Inc.). The solid phase immunosorbant assay used to screen column fractions was carried out by conventional procedure as previously described by Yeh et al., *Proc. Natl. Acad. Sci. USA* 84, 2317-2321 (1987).

45 Extraction of Growth Factor Activity from Acetone Powders. The acetone powders were prepared by conventional procedure as previously described by Porter et al., *J. Biol. Chem.* 263, 8989-8995 (1988), extracted with 40 ml/g of 0.9 M NaCl, 20 mM Tris-HCl (pH 8.0), 5 mM [ethylenebis(oxyethylenenitrilo)] tetra-acetic acid (EGTA), 0.15 mg/ml phenylmethylsulphonyl fluoride, 10 mM benzamidine, 0.1 mg/ml soybean trypsin inhibitor, and 1 μ g/ml aprotinin for 2 h at 4°C with agitation, and centrifuged at 10,000 x g for 30 min.

50 Heparin-Sepharose Chromatography. 6.9 liters of the soluble extract (34.5 g protein) was diluted to 20.7 liters in 900 mM NaCl, 20 mM Hepes, pH 7.0, loaded onto a 5 x 12.5 cm Heparin-Sepharose (Pharmacia) column at 4°C at a flow rate of 5 ml/min, washed in 10 column volumes of the starting buffer, 20 mM Hepes, pH 7.0, 900 mM NaCl, and eluted with 1160 ml of 20 mM Hepes, pH 7.0, 2 M NaCl, at a flow rate of 1 ml/min. The eluted fraction (2 M NaCl) was dialyzed against 20 mM MES, pH 6.0. Hepes = N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]. MES = 2[N-Morpholino]ethanesulfonic acid.

55 MonoS Chromatographic Separation of Mitogenic Activity. The active fraction (20 mM MES, pH 6.0) was loaded onto a MonoS HR 10/10 column (Pharmacia, FPLC) at 2.5 ml/min, washed with 10 column volumes of starting buffer, eluted with 12 ml (1.5 column volumes) of 20 mM MES, pH 6.0, 2 M NaCl, at a flow rate of 2.5 ml/min., diluted 1:4 4 with 20 mM MeS, pH 6.0 (final volume, 48 ml, 20 mM MES, pH 6.0, 500 mM NaCl), and loaded at 0.5 ml/min onto a MonoS HR 5/5 column (Pharmacia). Following loading, the column was washed with 10 column volumes of loading buffer and eluted with a 33 ml gradient from 500 mM NaCl to 2 M NaCl in 20 mM MES (pH 6.0). The column was further eluted with 2 M NaCl (10 ml) and the 1 ml fractions assayed in the NIH 3T3 mitogenic assay system. Each fraction also was assayed for bFGF immunoreactive proteins using a solid phase ELISA.

Microbore Cation Exchange Chromatography. The fractions (region A, Fig. 1, Mono HR 5/5 column) were pooled,

dialyzed against 2 mM Hepes, pH 7.0, concentrated (1 ml), and loaded on a 2.1 x 220 mm aquapore CX-300 weak cation exchange column (Brownlee Labs) at a flow rate of 200 μ l/min (Applied Biosystems, Inc., Model 130A Microbore HPLC system). The column was eluted with a 0-2 M NaCl gradient (15 ml, 20 mM Hepes, pH 7.0) at a flow rate of 200 μ l/min and fractions assayed in the NIH 3T3 assay system for mitogenic activity.

5 Reverse Phase Chromatographic Purification of bFGF. The fractions containing immunoreactive bFGF were loaded on a 2.1 x 220 nm Aquapore BU-300 C4 reverse phase column (Brownlee Labs, Applied Biosystems HPLC System), the column equilibrated with 100% water, 0.1% TFA, and eluted with a 15 ml gradient from 0-100% acetonitrile/0.1% TFA at a flow rate of 200 μ l/min. TFA = trifluoroacetic acid.

10 N-Terminal Amino Acid Sequencing. Fractions were analyzed by repetitive sequence analysis on a gas phase sequencer (Model 470A, Applied Biosystems, Inc.) essentially as described by Hunkapiller et al., *Meth. Enzymol.* 91, 399-412 (1983). The respective PTH-aa derivatives were identified using an Applied Biosystems Model 120A PTH analyzer. The initial yields on sequencing both proteins were 185 and 2475 pmoles for the bFGF and HBGF-8, respectively. Recoveries averaged greater than 95% for both sequences.

15 **RESULTS**

20 Purification of Heparin-Binding Growth Factor-B (HBGF-8). The purification scheme for bovine uterine HBGF-8 is shown in Table 1. 1.2 kg of frozen bovine uterus yielded 18 μ g of nearly pure HBGF-8 with an intrinsic specific activity in an NIH 3T3 cell mitogen assay of 5000 dpm/ng. Comparable values for aFGF and bFGF were 4090 and 9000 dpm/ng, respectively. Ten μ g bFGF per 1.2 kg wet weight of bovine uterus was also obtained (see alternative method, above). An overall recovery of 0.4% of HBGF-B activity with a 6944 fold purification was obtained; because uterus contains other growth factors active in mitogenic assays using NIH 3T3 cells as targets, the recovery is grossly underestimated due to the initial inclusion of all growth factors active against NIH 3T3 cells in assay of crude extracts of bovine uterus.

25 The MonoS HR 5/5 column used to further separate the mitogenic proteins binding to Heparin-Sepharose is shown in Fig. 1. bFGF immunoreactivity and mitogenic activity are shown. bFGF eluted at 0.8 M NaCl; it is both immunoreactive and a potent mitogen in the NIH 3T3 assay system. Importantly, significant activity bound to MonoS more tightly than bFGF; this data first suggested the presence of a novel growth factor.

30 The fractions indicated by the solid bar, region A, were pooled, dialyzed, concentrated and loaded onto the microbore CX-300 cation exchange column for elution with a 0.0-2.0 M NaCl gradient. The mitogenic activity identified (Fig. 2) corresponded to a 17 kD protein when analyzed in SDS-PAGE gels under reducing conditions. The appearance of the 17.5 kD bFGF and the novel 17 kD HDGF-8 by SDS-PAGE are shown in Fig. 3.

35 N-Terminal Amino Acid Sequencing. The separation of bFGF from HBGF-8 was pursued by N-terminal amino acid sequencing. The bFGF was further purified by C4 reverse phase chromatography to yield a single silver-stainable band at 17 kD on SDS-PAGE. N-terminal amino acid sequencing revealed N-terminal sequences for the bovine uterus bFGF proteins as shown in Table II. The N-terminal sequence confirmed that there is an N-terminal 2 amino acid extension beyond the first methionine sequenced (at position 3) which, during initial purifications of bFGF, was thought to be the site of initiation [Abraham et al., *EMBO J.* 5, 2523-2528 (1986)]. This N-terminal sequence obtained corresponded precisely with the N-terminal amino acid sequence for human placental bFGF [Sommer et al., *Biochem. Biophys. Res. Commun.* 144, 543-550 (1987)]. Alanine-2 in the bovine uterus bFGF sequence is substituted for threonine-2 in the human bFGF sequence.

40 A novel 25 amino acid N-terminal sequence was then obtained for HBGF-8, as shown in Table II, confirming the separation of two high affinity heparin-binding growth factors from bovine uterus. The sequence is extremely rich in lysines (7 of the first 12 amino acids). A search of both NBRF and GENBANK data bases failed to reveal sequence homology to any known protein or gene product. Thus, although bFGF and HBGF-8 elute nearly together from various columns and migrate nearly identically by SDS-PAGE, each may be separated from the other and each have different N-terminal amino acid sequence.

45 HBGF-8 is both a potent mitogen and abundant in uterus. It has an intrinsic specific activity in the NIH 3T3 assay system which is roughly equal within methods of assay to aFGF and bFGF. aFGF could not be detected among the growth factors in bovine uterus which bound to Heparin-Sepharose at 900 mM NaCl. The 25 amino acid N-terminal sequence of HBGF-8 has not been identified in any of the seven members of the FGF family; aFGF [Gimenez-Gallego, *supra.*], bFGF [Sommer et al., *supra.*], int-2 [Dickson and Peters, *supra.*], hst [Yoshida et al., *supra.*], FGF-5 [Zhan et al., *supra.*], FGF-5 [Marcis et al., *supra.*], FGF-6 [Finch et al., *supra.*].

50 HBGF-8 was purified with aqueous columns with pH values between 6.0 and 7.0. About 18 μ g/kg wet weight of tissue of HBGF-8 and ~ 10 μ g/kg wet weight tissue of bFGF were recovered. However, it is likely that there may be more HBGF-8 in uterus than 18 μ g/kg if all the activity which binds more tightly than bFGF in Fig. 1 is due to HBGF-8. Recently, aFGF has been shown to be more abundant than formerly thought with a tissue level of 500 μ g/kg in heart [Sasaki et al., *J. Biol. Chem.* 264, 17606-17612 (1989)].

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TABLE I. HBGF-8 purification scheme

	Total protein (mg)	Volume (ml)	Mitogenic activity* (dpmx10 ⁶)	Specific activity\$	Degree purification	Yield (%)
Solubilize acetone extract	†34,500	6,900	25,000	0.72	0	100
900-2000mM elution off heparin-sepharose	23	1,160	2,900	126	175	11.6
0-2000mM elution off Monos HR 10/10	3	12	433	144	200	1.7
500-1000mM elution off Monos HR 5/5	0.2	10	250	1250	1736	1.0
0-2000mM elution off CX-300 cation exchange column	0.018	0.6	90	5000	6944	0.4

*NIH 3T3 cell assay

\$units of specific activity are dpmx10⁶/mg protein

†1.2Kg frozen bovine uterus yields 207g acetone insoluble powder, which on dissolving in 900M NaCl, 50mM Hepes pH 7.0, produced solution containing 34.5g of protein.

TABLE III. Bovine uterus bFGF and HBGF-8 N-terminal sequence

bFGF

80% GLY-ALA-MET-ALA-ALA-GLY-SER-ILE-THR-THR-LEU-PRO-ALA-LEU-

10% MET-ALA-ALA-GLY-SER-ILE-THR-THR-LEU-PRO-ALA-LEU-

10% PRO-ALA-LEU-

HBGF-8

95% GLY-LYS-LYS-GLU-LYS-PRO-GLU-LYS-LYS-VAL-LYS-SER-ASP-

15 CYS-GLY-GLU-TRP-GLN-TRP-SER-VAL-CYS-VAL-PRO.

EXAMPLE 2

Male term placenta was flash-frozen as 1 cm dice in liquid nitrogen and stored at -70°C. Total cellular and poly(A) + RNA was prepared from placenta according to the conventional procedure of Chirgwin et al., Biochemistry 18, 5294-5299 (1979), as modified by Sadler et al., Proc. Natl. Acad. Sci. USA 82, 6394-6398 (1985). cDNA was synthesized and constructed in λgt11 arms and packaged by the published method of Ye et al., J. Biol. Chem. 262, 3710-3725 (1987). Approximately 1 x 10⁶ clones from the human placenta cDNA library were screened with a bovine cDNA probe. The screening procedure was as follows: the recombinant phages were replicated onto nitrocellulose filters and hybridized overnight at 42°C with random primer-labeled bovine cDNA probe using [α -³²P] dCTP (3,000 Ci/mmol, NEN), in 50% formamide, 50 mM sodium phosphate (pH 7.0), 5x Denhardt's Solution, 0.1 mg/ml purified tRNA and 5x standard saline citrate (SSC) (1x SSC is 150 mM sodium chloride, 15 mM sodium citrate). Filters were washed with 0.2x SSC and 0.1% SDS at 68°C and exposed to Kodak X-Omat AR film. After tertiary screening, a positive cDNA clone was subcloned into Bluescript (Stratagene). The nucleotide sequences were obtained by the well-known dideoxy chain termination method of Sanger et al., Proc. Natl. Acad. Sci. USA 74, 5463 (1977), with Sequenase version 2.0 (U.S. Biochemicals). The sequences were obtained by sequencing both strands of DNA twice with different primers.

The human HBGF-8 cDNA sequence revealed an open reading frame which encodes a putative 168 amino acid polypeptide with a calculated molecular weight of 18,942 daltons. A 25 amino acid sequence, which was identical to the experimentally determined N-terminal sequence of purified bovine HBGF-8, began 32 amino acids downstream of the proposed initiation codon. It also shows a hydrophobic N-terminal region of 32 amino acid residues which is believed to be a leader sequence. Comparison of predicted amino acid sequences of human and bovine HBGF-8 showed that HBGF-8 is highly conserved. Of 168 amino acid residues, 163 are identical.

Standard biochemical nomenclature is used herein in which the nucleotide bases are designated as adenine (A); thymine (T); guanine (G); and cytosine (C). Corresponding nucleotides are, for example, deoxyadenosine-5'-triphosphate (dATP). As is conventional for convenience in the structural representation of a DNA nucleotide sequence, only one strand is usually shown in which A on one strand connotes T on its complement and G connotes C.

Amino acids are shown herein by standard three letter abbreviations as follows:

Abbreviated Designation	Amino Acid
Ala	Alanine
Cys	Cysteine
Asp	Aspartic acid
Glu	Glutamic acid
Phe	Phenylalanine
Gly	Glycine
His	Histidine
Ile	Isoleucine
Lys	Lysine
Leu	Leucine

(continued)

Abbreviated Designation	Amino Acid
Met	Methionine
Asn	Asparagine
Pro	Proline
Gln	Glutamine
Arg	Arginine
Ser	Serine
Thr	Threonine
Val	Valine
Trp	Tryptophan
Tyr	Tyrosine

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Claims

20 1. A human placenta derived heparin-binding growth factor of 168 amino acids having the following amino acid sequence:

25 Met Gln Ala Gln Gln Tyr Gln Gln Gln Arg Arg Lys Phe Ala Ala 15
 Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala 30
 Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser 45
 Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly 60
 Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu 75
 Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn 90
 Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala 105
 Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly 120
 Ser Leu Lys Arg Ala Leu His Asn Ala Glu Cys Gln Lys Thr Val 135
 Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Pro Lys Pro Gln 150
 40 Ala Glu Ser Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys 165
 Met Leu Asp 168

45 2. A DNA sequence consisting of a sequence encoding human heparin binding growth factor of 168 amino acids having the following amino acid sequence:

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5	Met Gln Ala Gln Gln Tyr Gln Gln Arg Arg Lys Phe Ala Ala	15
	Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala	30
	Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser	45
10	Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly	60
	Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu	75
	Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn	90
15	Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala	105
	Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly	120
	Ser Leu Lys Arg Ala Leu His Asn Ala Glu Cys Gln Lys Thr Val	135
20	Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Pro Lys Pro Gln	150
	Ala Glu Ser Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys	165
	Met Leu Asp	168

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3. The cDNA of human heparin-binding growth factor having the following nucleotide sequence:

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	GTCAAAGGCA GGATCAGGTT CCCCGCCTTC CAGTCCAAA ATCCCGCCAA	50
5	GAGAGCCCCA GAGCAGAGGA AAATCCAAAG TGGAGAGAGG GGAAGAAAGA	100
	GACCAGTGAG TCATCCGTCC AGAAGGCGGG GAGAGCAGCA CGGGCCCAAG	150
	CAGGAGCTGC AGCGAGCCGG GTACCTGGAC TCAGCGGTAG CAACCTCGCC	200
10	CCTTGCAACA AAGGCAGACT GAGCGCCAGA GAGGACGTTT CCAACTCAA	250
	AATGCAGGCT CAACAGTACC AGCAGCAGCG TCGAAAATTT GCAGCTGCCT	300
	TCTTGGCATT CATTTCATA CTGGCAGCTG TGGATACTGC TGAAGCAGGG	350
15	AAGAAAGAGA AACCAAGAAAA AAAAGTGAAG AAGTCTGACT GTGGAGAATG	400
	GCAGTGGAGT GTGTGTGTGC CCACCAAGTGG AGACTGTGGG CTGGGCACAC	450
20	GGGAGGGCAC TCGGACTGGA GCTGAGTCCA AGCAAACCAT GAAGACCCAG	500
	AGATGTAAGA TCCCCCTGCAA CTGGAAGAAG CAATTGGCG CGGAGTGC	550
	ATACCAGTTC CAGGCCTGGG GAGAATGTGA CCTGAACACA GCCCTGAAGA	600
25	CCAGAACTGG AAGTCTGAAG CGAGCCCTGC ACAATGCCGA ATGCCAGAAG	650
	ACTGTCACCA CTCCCAAGCC CTGTGGCAAA CTGACCAAGC CCAAACCTCA	700
	AGCAGAAATCT AAGAAGAAGA AAAAGGAAGG CAAGAAACAG GAGAAGATGC	750
30	TGGATTAAAA GATGTCACCT GTGGAACATA AAAAGGACAT CAGCAAACAG	800
	GATCAGTTAA CTATTGCATT TATATGTACC GTAGGCTTTG TATTCAAAAAA	850
35	TTATCTATAG CTAAGTACAC AATAAGCAA AACAAAAAGA AAAAAAAA	900
	AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA	950
	AAAAAAAAAA A	961

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Patentansprüche

45 1. Aus einer menschlichen Plazenta abgeleiter Heparin-bindender Wachstumsfaktor aus 168 Aminosäuren mit der nachfolgenden Aminosäuresequenz:

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	Met Gln Ala Gln Gln Tyr Gln Gln Gln Arg Arg Lys Phe Ala Ala	15
5	Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala	30
	Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser	45
	Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly	60
10	Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu	75
	Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn	90
	Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala	105
15	Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly	120
	Ser Leu Lys Arg Ala Leu His Asn Ala Glu Cys Gln Lys Thr Val	135
	Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Pro Lys Pro Gln	150
20	Ala Glu Ser Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys	165
	Met Leu Asp	168

25 2. DNA-Sequenz,
bestehend aus einer Sequenz, die einen menschlichen Heparin-bindenden Wachstumsfaktor aus 168 Aminosäuren mit der nachfolgenden Aminosäuresequenz kodiert:

30	Met Gln Ala Gln Gln Tyr Gln Gln Gln Arg Arg Lys Phe Ala Ala	15
	Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala	30
	Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser	45
35	Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly	60
	Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu	75
	Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn	90
40	Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala	105
	Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly	120
45	Ser Leu Lys Arg Ala Leu His Asn Ala Glu Cys Gln Lys Thr Val	135
	Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Pro Lys Pro Gln	150
	Ala Glu Ser Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys	165
50	Met Leu Asp	168

3. cDNA von menschlichem Heparin-bindendem Wachstumsfaktor mit der nachfolgenden Nukleotidsequenz:

5	GTCAAAGGCA GGATCAGGTT CCCCGCCTTC CAGTCCAAAA ATCCCGCCAA	50
	GAGAGCCCCA GAGCAGAGGA AAATCCAAAG TGGAGAGAGG GGAAGAAAGA	100
	GACCACTGAG TCATCCGTCC AGAAGGCCGG GAGAGCAGCA GCGGCCCAAG	150
	CAGGAGCTGC AGCGAGCCGG GTACCTGGAC TCAGCGGTAG CAACCTCGCC	200
10	CCTTGCAACA AAGGCAGACT GAGGCCAGA GAGGACGTTT CCAACTCAA	250
	AATGCAGGCT CAACAGTACC AGCAGCAGCG TCGAAAATTT GCAGCTGCCT	300
	TCTTGGCATT CATTTCATA CTGGCAGCTG TGGATACTGC TGAAGCAGGG	350
15	AAGAAAGAGA AACCAAGAAA AAAAGTGAAG AAGTCTGACT GTGGAGAATG	400
	GCAGTGGAGT GTGTGTGTGC CCACCAGTGG AGACTGTGGG CTGGGCACAC	450
	GGGAGGGCAC TCGGACTGGA GCTGAGTCCA AGCAAACCAT GAAGACCCAG	500
20	AGATGTAAGA TCCCCTGCAA CTGGAAGAAG CAATTGGCG CGGAGTGCAA	550
	ATACCAGTTC CAGGCCTGGG GAGAATGTGA CCTGAACACA GCCCTGAAGA	600
	CCAGAACTGG AAGTCTGAAG CGAGCCCTGC ACAATGCCGA ATGCCAGAAG	650
	ACTGTCACCA CTCCCAAGCC CTGTGGCAAA CTGACCAAGC CCAAACCTCA	700
	AGCAGAATCT AAGAAGAAGA AAAAGGAAGG CAAGAACACAG GAGAAGATGC	750
30	TGGATTAAAA GATGTCACCT GTGGAACATA AAAAGGACAT CAGCAAACAG	800
	GATCAGTTAA CTATTGCATT TATATGTACC GTAGGCTTG TATTCAAAAA	850
	TTATCTATAG CTAAGTACAC AATAAGCAA AACAAAAAGA AAAAAAAA	900
35	AAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA	950
	AAAAAAA A	961

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Revendications

1. Facteur de croissance fixant l'héparine, dérivé du placenta humain, constitué de 168 amino-acides, avant la séquence d'amino-acides suivante :

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	Met Gln Ala Gln Gln Tyr Gln Gln Gln Arg Arg Lys Phe Ala Ala	15
5	Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala	30
	Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser	45
	Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly	60
10	Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu	75
	Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn	90
	Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala	105
15	Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly	120
	Ser Leu Lys Arg Ala Leu His Asn Ala Glu Cys Gln Lys Thr Val	135
	Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Pro Lys Pro Gln	150
20	Ala Glu Ser Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys	165
	Met Leu Asp	168

2. Séquence d'ADN consistant en une séquence codant pour le facteur de croissance fixant l'héparine, d'origine humaine, constitué de 168 amino-acides, ayant la séquence d'amino-acides suivante :

25	Met Gln Ala Gln Gln Tyr Gln Gln Gln Arg Arg Lys Phe Ala Ala	15
	Ala Phe Leu Ala Phe Ile Phe Ile Leu Ala Ala Val Asp Thr Ala	30
30	Glu Ala Gly Lys Lys Glu Lys Pro Glu Lys Lys Val Lys Lys Ser	45
	Asp Cys Gly Glu Trp Gln Trp Ser Val Cys Val Pro Thr Ser Gly	60
35	Asp Cys Gly Leu Gly Thr Arg Glu Gly Thr Arg Thr Gly Ala Glu	75
	Cys Lys Gln Thr Met Lys Thr Gln Arg Cys Lys Ile Pro Cys Asn	90
	Trp Lys Lys Gln Phe Gly Ala Glu Cys Lys Tyr Gln Phe Gln Ala	105
40	Trp Gly Glu Cys Asp Leu Asn Thr Ala Leu Lys Thr Arg Thr Gly	120
	Ser Leu Lys Arg Ala Leu His Asn Ala Glu Cys Gln Lys Thr Val	135
45	Thr Ile Ser Lys Pro Cys Gly Lys Leu Thr Lys Pro Lys Pro Gln	150
	Ala Glu Ser Lys Lys Lys Lys Glu Gly Lys Lys Gln Glu Lys	165
	Met Leu Asp	168

50 3. ADNc d'un facteur de croissance humain fixant l'héparine ayant la séquence de nucléotides suivante :

	GTCAAAGGCA GGATCAGGTT CCCCCGCCTTC CAGTCCAAAA ATCCCGCCAA	50
5	GAGAGCCCCA GAGCAGAGGA AAATCCAAAG TGGAGAGAGG GGAAGAAAGA	100
	GACCAGTGAG TCATCCGTCC AGAAGGCGGG GAGAGCAGCA GCGGCCCAAG	150
	CAGGAGCTGC AGCGAGCCGG GTACCTGGAC TCAGCGGTAG CAACCTCGCC	200
10	CCTTGCACA AAGGCAGACT GAGGCCAGA GAGGACGTTT CCAACTCAAA	250
	AATGCAGGCT CAACAGTACC AGCAGCAGCG TCGAAAATT GCAGCTGCCT	300
15	TCTTGGCATT CATTTCATA CTGGCAGCTG TGGATACTGC TGAAGCAGGG	350
	AAGAAAGAGA AACCAGAAAA AAAAGTGAAG AAGTCTGACT GTGGAGAATG	400
	GCAGTGGAGT GTGTGTGTGC CCACCAGTGG AGACTGTGGG CTGGGCACAC	450
20	GGGAGGGCAC TCGGACTGGA GCTGAGTCCA AGCAAACCAT GAAGACCCAG	500
	AGATGTAAGA TCCCCTGCAA CTGGAAGAAG CAATTTGGCG CGGAGTGC	550
	ATACCAGTTC CAGGCCTGGG GAGAATGTGA CCTGAACACA GCCCTGAAGA	600
25	CCAGAACTGG AAGTCTGAAG CGAGCCCTGC ACAATGCCGA ATGCCAGAAG	650
	ACTGTCACCA CTCCCAAGCC CTGTGGCAAA CTGACCAAGC CCAAACCTCA	700
30	AGCAGAAATCT AAGAAGAAGA AAAAGGAAGG CAAGAAACAG GAGAAGATGC	750
	TGGATTAAAA GATGTCACCT GTGGAACATA AAAAGGACAT CAGCAAACAG	800
	GATCAGTTAA CTATTGCATT TATATGTACC GTAGGCTTG TATTCAAAAA	850
35	TTATCTATAG CTAAGTACAC AATAAGCAAA AACAAAAAGA AAAAAAAA	900
	AAAAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA	950
40	AAAAAAAAAA A	961

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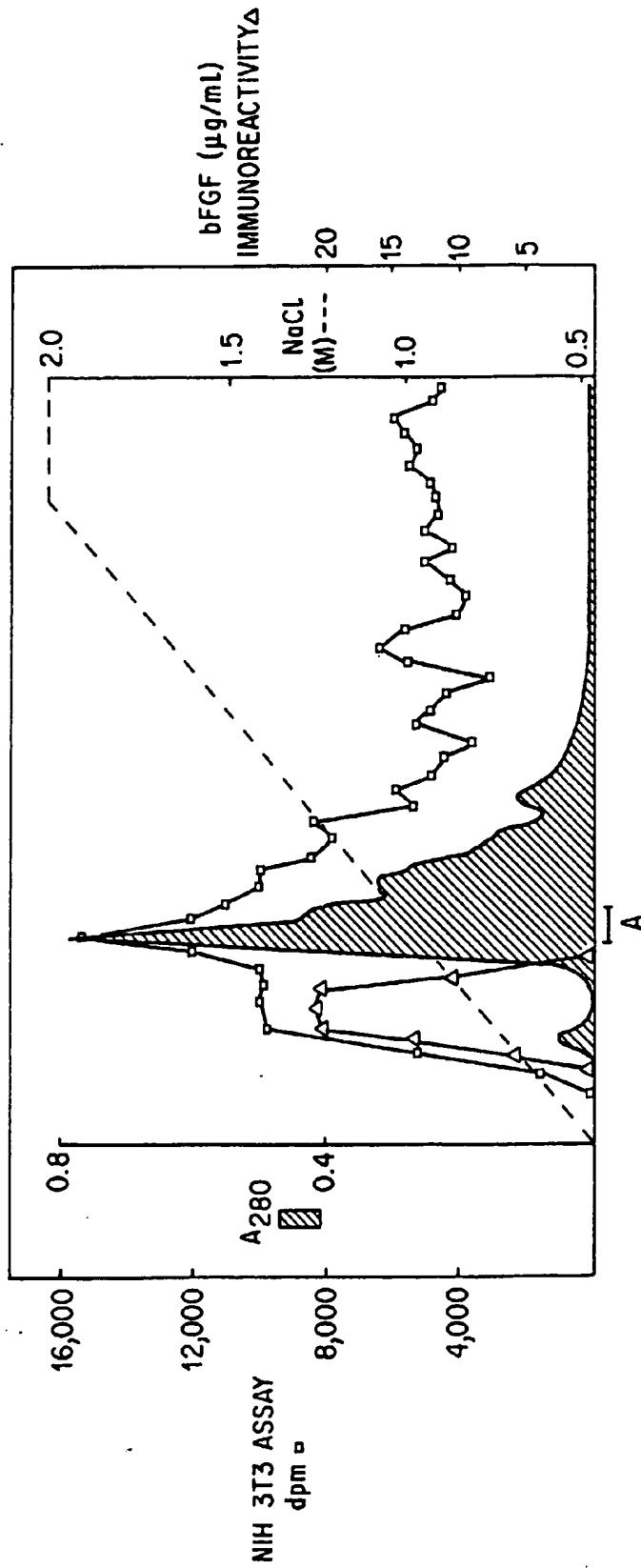


FIG. 1

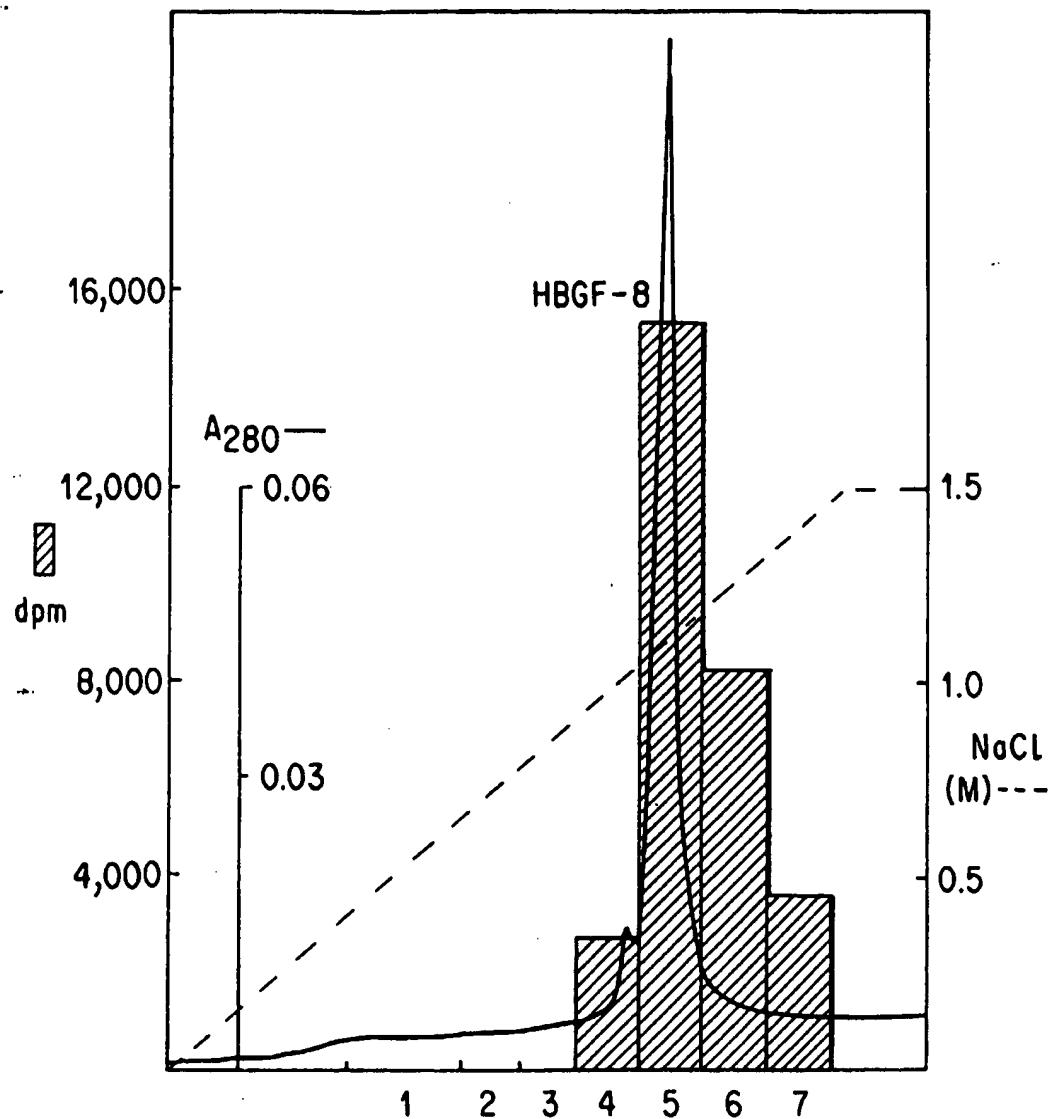


FIG. 2

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FIG. 3